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Comparison of the Toxicity of Acrylamide Cyclophosphamide, Chlrodecone, and Diethylstilbestrol in Chinese Hamster Ovary (CHO) Cells with Their Toxicity In vivo

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ABSTRACT

In order to compare *in vitro* toxicity with *in vivo* toxicity, four chemicals that have been tested in the *in vivo/in vitro* toxicological screen proposed by the Health Effects Research Laboratory, EPA were tested in a Chinese Hamster Ovary (CHO) cytotoxicity assay. Viability index, cellular ATP concentration and rate of protein synthesis were decreased by exposure to acrylamide (AC), chlordecone (CH), diethylstilbestrol (DES), and cyclophosphamide (CY) ($P < 0.00001$). All of these chemcials were active in the CHO cytotoxicity assay. The *in vitro* toxicity rankings (from most to least toxic) based either on cellular ATP concentration or rate of protein synthesis were identical: DES $\cong$ CH > AC $\cong$ CY. The toxicity ranking based on viability index was: DES $\cong$ CH > AC > CY. All of these chemicals were toxic *in vivo* following 10 days of repeated dosing. The *in vivo* toxicity rankings (from most to least toxic) based either on mortality or on liver weight were identical: CY > AC > DES (unable to rank CH since highest concentration tested produced no change from control). The toxicity ranking for body weight at sacrifice was: CH $\cong$ CY > AC (unable to rank DES since lowest concentration tested reduced body weight). The *in vitro* toxicity rankings did not correlate with *in vivo* toxicity rankings based on death, body weight, or liver weight. These results demonstrate the potential usefulness of the CHO cell assay to detect biological activity but raise a question concerning the usefulness of this assay to predict *in vivo* toxicity rankings. The limited number of chemicals that we tested does not allow us to answer this question.

INTRODUCTION

Acrylamide (AC), cyclophosphamide (CY), chlordecone (CH), and diethylstilbestrol (DES) have recently been tested in a proposed *in vivo/in vitro* toxicological screen (1). Included in this toxicological screen are biological endpoints to detect neurotoxicity, reproductive toxicity, teratology, mutagenicity, immunotoxicity, and general toxicity. In the toxicological screen male F344 rats are gavaged daily for 10 consecutive days with the test material; the full toxicological screen requires the use of 86 male rats and 48 female rats. This screen was developed to evaluate potentially hazardous complex waste mixtures and process stream residuals (1).

An integral part of the proposed toxicological screen is another *in vivo* test protocol, the Prescreen. The Prescreen would be used to screen samples for biological activity and to prioritize them for further *in vivo* evaluation in the proposed *in vivo/in vitro* toxicological screen. The current Prescreen protocol calls for gavaging male rats for 10 days with the test material and requires 20 rats (1).

To screen and prioritize samples for further testing, *in vitro* test systems offer potential advantages when compared to *in vivo* test systems. These advantages include: the decreased time required to test a chemical; the decreased cost; the decreased number of experimental animals; and, the decreased amount of test material required.

Chinese hamster ovary (CHO) cells have been used extensively to monitor genetic damage following chemical treatment (2,3,4,5) CHO cells have been used

also to evaluate the cellular toxicity of chemicals, chemical mixtures, and environmental pollutants (6,7,8,9,10,11,12,13) including fly ash and coal-related particles, coal oil, effluent from textile mills, oil refining waste, herbicide manufacturing waste, and coke plant waste.

Data from various toxicity assays using CHO cells have been reported to correlate well with *in vivo* toxicity data. Hsie *et al.* and Tan *et al.* evaluated the toxicity of 16 metal salts in a CHO assay by measuring cloning efficiency subsequent to chemical treatment (14,15). They found good correlation between the toxicity of these compounds in CHO cells and the LD_{50} values determined using the intraperitoneal route of adminstration for Balb/c mice, as well as the LC_{50} values for male *Drosophila melanogaster* (14,15,16). Garrett and Lewtas (10) ranked the cellular toxicity of 19 chemicals based on their ability to inhibit DNA synthesis in CHO cells and found good correlation between these *in vitro* toxicity rankings and *in vivo* toxicity rankings based on published rat LD_{50} values, determined using the oral route of administration. These 19 chemicals included metallic compounds, phenols, chlorinated alkanes, and aromatics.

The CHO cytotoxicity assay described by Garrett *et al.* (8) and Garrett and Lewtas (10) is relatively simple and can be done in a short period of time ($\cong$ 2 days). Given the simplicity of this assay and the reported correlations between *in vitro* CHO cell data and *in vivo* toxicity data, it appeared feasible to examine the suitability of the CHO cytotoxicity assay to screen samples for biological activity and to prioritize them for evaluation *in vivo*. We used the CHO cytotoxicity assay to test the four chemicals that have been tested in the proposed *in vivo*/*in vitro* toxicological screen. In this report, we describe and compare the toxicity rankings obtained in either system.

MATERIALS AND METHODS

Chemicals

Acrylamide and DES were obtained from Aldrich Chemical Co.; cyclophosphamide from Sigma Chemical Co.; and chlordecone from the Pesticides and Industrial Chemicals Repository, Environmental Protection Agency. Acrylamide was stored in the dark.

In Vitro Experiments

CHO cells (American Type Culture Collection) were maintained in Ham's F-12 medium (GIBCO, Long Island, N.Y.) supplemented with 5% fetal bovine serum (virus and mycoplasma screened) (Flow Laboratories, McLean, Va.). The cells were incubated at 37°C and subcultured twice weekly using 0.25% trypsin (GIBCO, Long Island, N.Y.).

For toxicity testing, 1×10^6 cells were incubated in 2 ml of Ham's F-12 medium supplemented with 10% fetal bovine serum, 50 units/ml pencillin, 50 ug/ml streptomycin, and 50 ug/ml kanamycin, in six-well cluster dishes. Each experiment consisted of 5 replicate cultures of control cells and 3 replicate cultures both of DMSO controls (when appropriate) and of each test concentration. The chemical-media mixtures were prepared immediately prior to exposure. Acrylamide and cyclophosphamide were dissolved directly in the culture medium. Chlordecone and DES were dissolved in DMSO before they were added to the culture

medium. The concentration of DMSO was held constant in each experiment and did not exceed 0.7% in any experiment. After the test chemical was added, the cells were placed on a rocker platform and incubated for 20 hours at 37°C and 5% CO_2. Dose-ranging experiments were conducted, followed by two replicate experiments with each
chemical.

Twenty hours after adding the test chemical, cell numbers and viability were determined optically using a hemacytometer and trypan blue dye. Viability index was calculated by dividing the number of treated cells by the average number of control cells and multiplying the resulting fraction by the percent viability of the treated cells (17). The concentration of cellular ATP was measured using the luciferin-luciferase assay (8). Separate sets of cultures were used to measure the rate of incorporation of ^{3}H-leucine into cellular protein using the method of Byfield and Scherbaum (18) as modified by Garrett and Lewtas (10).

The data were analyzed using the procedures of analysis of variance (19). Prior to the analyses, the data were subjected to Bartlett's test for homogeneity of variances (20). The method of Tukey-Kramer was used to determine the lowest tested concentration of chemical at which a significant difference from the control value occurred (19). The 0.05 level of significance was used as the criterion of significance.

In vivo Experiments

Male Fischer-344 rats (Charles River Laboratories, Kingston, N.Y.) that were 60-days-old on arrival were used. The rats were housed in groups of 2 on heat-treated pine-shavings and were fed Purina Rodent Lab Chow (Richmond, Ind.) and tap water ad libitum. The room in which the rats were housed was illuminated for 12 hours each day beginning at 6 A.M. The room temperature was 22 ± 2°C and the relative humidity was 50 ± 10%. Rats were housed under these conditions for a minimum of 7 days before the experiments were begun.

Rats were exposed daily by gavage for ten consecutive days, in treatment groups of ten. Each chemical was tested at 5 concentrations with a vehicle control and a cage control. Acrylamide was dissolved in water and administered at dosage rates of 0, 0.05, 0.11, 0.21, 0.42, and 0.84 mmol/kg/day. Chlordecone was dissolved in corn oil and administered at 0, 0.001, 0.002, 0.005, 0.01, and 0.02 mmol/kg/day. Cyclophosphamide was dissolved in water and administered at 0, 0.005, 0.01, 0.02, 0.04, and 0.09 mmol/kg/day. DES was suspended in corn oil and administered at 0.12, 0.23, 0.46, 0.93, and 1.86 mmol/kg/day.

Forty-eight hours after the last treatment, the rats were weighed, anesthetized with ether, and killed by exsanguination from the abdominal aorta. The livers were removed and weighed.

The data were analyzed using the procedures of analysis of variance (21) When the results of this analysis indicated statistically significant differences between groups, Dunnett's test was used to determine those tested concentrations at which significant differences from the vehicle control occurred. The 0.05 level of probability was used as the criterion of significance.

RESULTS

In vitro Experiments (CHO cells)

Data for viability index, the concentration of cellular ATP, and the rate of protein synthesis are given for each chemical in Tables 1-4. The cultures that received only DMSO (data not shown) did not differ significantly ($P > 0.05$) from the control cultures; therefore, the data from the DES and the chlordecone treated cells were compared to the controls.

Viability index, concentration of cellular ATP, and rate of protein synthesis were decreased by exposure to acrylamide ($P < 0.00001$), to DES ($P < 0.00001$), to chlordecone ($P < 0.00001$), and to cyclophosphamide ($P < 0.00001$). The magnitude of the effect of chemical treatment on these endpoints was dependent on the concentration of each of the four chemicals. The lowest tested concentration of each chemical at which a significant difference from the control occurred in both replicated experiments is listed in Table 5. The lowest concentration at which a significant decrease from control occurred for any of the three endpoints was 0.01 mM DES, 0.02 mM chlordecone, 2.50 mM acrylamide, and 5.00 mM cyclophosphamide. The toxicity rankings for each endpoint based on the lowest significant concentrations are given in Table 6.

In Vivo Experiments

Mortality, body weight, and liver weight data for each chemical are given in Tables 7-10. Exposure to 0.09 mmol/kg/day cyclophosphamide, 0.84 mmol/kg/day acrylamide, and 1.86 mmol/kg/day DES resulted in 70%, 30%, and 10% mortality, respectively. No deaths occurred at 0.02 mmol/kg/day chlordecone, the highest concentration tested.

Exposure to each chemical resulted in decreased body weight at sacrifice ($P < 0.05$). The mean body weight at sacrifice was 60, 84, and 73% of the vehicle control after exposure to the highest concentrations of acrylamide, chlordecone, and cyclophosphamide, respectively. Exposure to all concentrations of DES resulted in decreased body weight at sacrifice; mean body weights ranged from 68% to 80% of the vehicle control with the lowest body weights (68% of control) occuring in the lowest and the highest treatment groups.

Liver weight was decreased by exposure to acrylamide ($P < 0.05$) and cyclophosphamide ($P < 0.05$). A statistically significant increase in liver weight occurred at 0.01 mmol/kg/day chlordecone ($P < 0.05$); this change was of unknown biological significance since an increase was not apparent at 0.02 mmol/kg/day, the highest concentration of chlordecone tested. A statistically significant decrease occurred at 0.12 mmol/kg/day DES ($P < 0.05$); this change was also of unknown biological significance since changes were not apparent at any of the higher concentrations of DES.

Table 11 summarizes the in vivo results showing, for each endpoint, the lowest tested concentration of each chemical at which a significant difference from control occurred. The lowest concentration at which a significant difference from control occurred for any endpoint was 0.02 mmol/ kg/day chlordecone, 0.04 mmol/kg/day cyclophosphamide, and 0.84 mmol/kg/day acrylamide. Because the lowest concentration of DES tested (0.12 mmol/kg/day) resulted in decreased body weight, the lowest concentration at which a significant decrease from control would occur probably lies below this concentration. The toxicity rankings for each endpoint based on the lowest significant concentrations are given in Table 12.

TABLE 1.

Effect of Exposure to Acrylamide on Viability Index, Concentration of Cellular ATP, and Rate of Protein Synthesis in Chinese Hamster Ovary Cells. N = 10 for controls and 6 for treatments.

Acrylamide (mM)	0	1.0	2.5	5.0	7.5	10.0	12.5	15.0	20.0
Viability index	99.1[a]	87.8	62.9*	46.8*	44.2*	34.8*	26.5*	16.6*	6.4*
	6.8	11.0	9.8	9.0	3.7	6.6	5.8	3.5	2.5
ATP concentration,	100.0	102.8	102.8	96.6	73.8	53.0*	37.3*	18.6*	4.2[b]*
% of control	9.4	11.2	14.2	15.5	12.2	9.9	7.5	3.6	1.1
Protein sythesis,	100.2[c]	102.0[b]	92.3[b]	71.3*	52.7[b]*	33.7*	21.2[b]*	15.5*	10.3*
% of control	5.4	4.1	10.4	3.1	3.2	4.1	1.3	1.0	2.4

[a] mean ∓ s.d.
[b] N = 5.
[c] N = 9.
* significantly different from controls: $P < 0.05$.

TABLE 2.

Effect of Exposure to Chlordecone on Viability Index, Concentration of Cellular ATP, and Rate of Protein Synthesis in Chinese Hamster Ovary Cells. N = 10 for controls and 6 for treatments.

Chlordecone (mM)	0	0.001	0.01	0.02	0.03	0.04	0.05	0.06	0.07
Viability index	99.3[a]	100.2	100.1	83.4	59.4*	51.7*	37.6*	21.3*	10.7*
	13.0	7.0	9.5	10.3	5.5	4.8	7.9	9.0	9.8
ATP concentration,	100.0	97.5	85.8	53.6*	38.5*	32.2*	22.1*	10.4*	6.1[b]*
% of control	9.6	6.6	11.6	13.6	5.0	2.1	8.4	7.8	5.0
Protein synthesis	100.0	99.1	96.0	78.9*	34.3*	18.8*	11.0*	5.3*	2.3*
% of control	2.7	3.1	3.0	5.6	7.4	3.4	2.1	2.5	1.7

[a] mean ∓ s.d.
[b] N = 3.
* significantly different from controls: $P < 0.05$.

TABLE 3.

Effect of Exposure to Cyclophosphamide on Viability Index, Concentration of Cellular ATP, and Rate of Protein Synthesis in Chinese Hamster Ovary Cells. N = 10 for controls and 6 for treatments.

Cyclophosphamide (mM)	0	1	5	10	15	20	25	50
Viability index	97.8[a]	90.1	77.8	63.5*	62.7*	46.1*	25.1*	0.5*
	11.3	16.1	14.5	4.1	12.2	9.7	16.7	0.4
ATP concentration,	100.0[b]	96.2	91.3[c]	72.4	54.2[c]*	33.5*	15.8[c]*	0.1*
% of control	11.7	18.6	13.8	8.3	4.8	4.8	8.0	0.1
Protein sythesis,	100.0	93.0	85.0[c]*	57.2*	30.7*	14.0*	3.1*	0.1[c]*
% of control	4.6	2.9	3.0	6.2	4.0	1.8	1.2	0.1

[a] mean ∓ s.d.
[b] N = 9; [c] N = 5.
* significantly different from controls: $P < 0.05$.

TABLE 4.

Effect of Exposure to DES on Viability Index, Concentration of Cellular ATP, and Rate of Protein Synthesis in Chinese Hamster Ovary Cells. N = 10 for controls and 6 for treatments.

DES (mM)	0	0.001	0.005	0.01	0.05	0.10	0.50	1.00
Viability index	99.1[a]	103.2	90.4	85.1	41.2*	32.4*	0.12*	0.0*
	12.7	9.1	6.7	6.8	2.7	7.6	0.2	0.0
ATP concentration,	100.0[b]	81.1	81.1	69.9*	49.7*	16.4*	0.0*	0.2*
% of control	13.4	11.9	16.2	14.0	2.2	5.7	0.0	0.2
Protein synthesis	100.0[b]	100.7[c]	95.8	89.7*	44.3[c]*	6.6[c]*	0.4*	0.3[c]*
% of control	3.5	2.3	3.7	3.7	3.7	2.6	0.6	0.4

[a] mean ∓ s.d.
[b] N = 9; [c] N = 5.
* significantly different from controls: $P < 0.05$.

TABLE 5.
The Lowest Concentration (mM) that Produced a Significant Difference from Controls in Chinese Hamster Ovary Cells.

	DES	CH	AC	CY
Viability index	0.05	0.03	2.50	10.00
ATP, % control	0.01	0.02	10.00	15.00
Protein synthesis, % control	0.01	0.02	5.00	5.00

TABLE 6.
Toxicity Ranking Based on the Lowest Significant Concentrations in Chinese Hamster Ovary Cells.

Viability index:	DES ≅ CH > AC > CY
ATP, % control:	DES ≅ CH > AC ≅ CY
Protein synthesis, % control:	DES ≅ CH > AC ≅ CY

TABLE 7.
Effect of Exposure to Acrylamide on Mortality, Body Weight, and Liver Weight in male F344 rats. N = 10.

Acrylamide (mmol/kg/day)	0	0.05	0.11	0.21	0.42	0.84
Mortality, %	0	0	0	0	0	30
Body weight at sacrifice, gm	223[a] 10.3	232 9.1	226 10.7	227 8.4	215 8.0	135[b]* 7.9
Liver weight, gm	9.94 0.73	10.42 0.63	10.45 0.67	10.72 0.70	10.33 0.76	4.29[b]* 0.65

[a] mean ± s.d.
[b] N = 7.
* significantly different from vehicle controls: $P < 0.05$.

TABLE 8.
Effect of Exposure to Chlordecone on Mortality, Body Weight, and Liver Weight in male F344 rats. N = 10.

Chlordecone (mmol/kg/day)	0	0.001	0.002	0.005	0.01	0.02
Mortality, %	0	0	0	0	0	0
Body weight at sacrifice, gm[a]	226[b] 6.9	228 6.1	229 3.9	230 4.2	227 7.7	190* 18.8
Liver weight, gm	9.82 0.57	9.83 0.54	10.39 0.87	10.51 0.43	11.36* 0.55	9.50 0.78

[a] Body weight data have been reported by Smialowicz et al. (22.)
[b] mean ± s.d.
* significantly different from vehicle controls: $P < 0.05$.

TABLE 9.
Effect of Exposure to Cyclophosphamide on Mortality, Body Weight, and Liver Weight in male F344 rats. N = 10.

Cyclophosphamide (mmol/kg/day)	0	0.005	0.01	0.02	0.04	0.09
Mortality, %	0	0	0	0	0	70
Body weight at sacrifice, gm[a]	232[b] 12.6	231 12.5	229 6.1	229 9.3	213* 9.6	169[c]* 12.2
Liver weight, gm	9.79 0.68	9.85 0.85	9.84 0.38	9.99 0.65	9.53 0.74	7.41[c]* 1.02

[a] Body weight data have been reported by Smialowicz et al.(22).
[b] mean ± s.d.
[c] N = 3.
* significantly different from vehicle controls: $P < 0.05$.

TABLE 10.
Effect of Exposure to DES on Mortality, Body Weight, and Liver Weight in male F344 rats. N = 10.

DES (mmol/kg/day)	0	0.12	0.23	0.46	0.93	1.86
Mortality, %	0	0	0	0	0	10
Body weight at sacrifice, gm	213[a] 10.5	145* 9.8	157* 12.3	163* 10.2	171* 7.7	144[b]* 17.9
Liver weight, gm	9.95 0.68	7.41* 0.99	9.58 1.27	10.51 0.81	11.14 0.73	10.26[b] 1.83

[a] mean ± s.d.
[b] N = 9.
* significantly from vehicle controls: $P < 0.05$.

TABLE 11.
The Lowest Concentration (mmol/kg/day) *in vivo* That Produced a Significant Difference from Control.

	DES	CH	AC	CY
Mortality, %	1.86	> 0.02	0.84	0.09
Body Weight at sacrifice, gm	< 0.12	0.02	0.84	0.04
Liver Weight, gm	> 1.86	> 0.02	0.84	0.09

TABLE 12.
Toxicity Rankings *in vivo* Based on the Lowest Significant Concentrations.

Mortality, %[1]	CY > AC > DES
Body weight at sacrifice, gm[2]	CH ≡ CY > AC
Liver weight, gm[1]	CY > AC > DES

[1] unable to rank CH since highest concentration tested did not result in significant change from control.
[2] unable to rank DES since lowest concentration tested resulted in significant change from control.

DISCUSSION

The four tested chemicals were active in the CHO cytotoxicity assay. All three endpoints (viability index, concentration of ATP, and protein synthesis) demonstrated comparable ability to detect the biological activity of these diverse chemicals. The results demonstrate the potential usefulness of an *in vitro* test system, such as the CHO cytotoxicity assay, to screen samples for biological activity.

The toxicity rankings *in vitro* did not appear to correlate well with *in vivo* toxicity rankings based either on mortality or on changes in body and liver weight following 10 days of repeated dosing. For example, based either on *in vivo* mortality or on liver weight changes, DES was less toxic than either acrylamide or cyclophosphamide, whereas based on all three *in vitro* endpoints, DES was more toxic than either acrylamide or cyclophosphamide. If one assumes that the ability of a test system to correctly rank chemicals according to their *in vivo* toxicity is a prerequisite for the use of the test system for prioritizing samples for *in vivo* testing, the utility of the CHO cytotoxicity assay is at question.

In a further effort to compare the *in vitro* data to *in vivo* data, we used the criteria used by Garrett and Lewtas (8) to compare toxicity data from the CHO assay with rat LD_{50} data. The *in vivo* and *in vitro* toxicity rankings developed using this method are given in Table 13. There was reasonable correlation between the *in vivo* and the *in vitro* rankings for DES and chlordecone, less correlation for acrylamide, and, no correlation for cyclophosphamide. Garrett and Lewtas based their rankings on the effective concentration required to produce a 50% reduction (EC_{50}) in DNA synthesis. DNA synthesis was no t measured in the present study, but Garrett and Lewtas reported that toxicity rankings based on DNA synthesis were similar to those based on protein synthesis.

It is interesting to note the discrepancy between the *in vitro* and the *in vivo* rankings for cyclophosphamide. Cyclophosphamide requires metabolic activation to exert cytotoxicity (23,24,25,26). CHO cells have been noted for their inability to transform those compounds that require metabolic activation before expression of toxicity (2). A major difficulty in using a continuous cell line to screen chemicals for toxicity is that many cell types in continuous culture have undergone dedifferentiation and have lost their metabolic activation

and detoxification capabilities (7). Various methods have been developed to more closely mimic the metabolic capability of the intact animal, including: the addition of liver homogenates from various animals, with rat liver homogenate being the most commonly used; the use of primary cells such as primary hepatoctyes as target cells; and, the use of co-cultivation techniques where the primary cell is used to metabolically activate the test chemical but another cell type is used as the target. The more closely the *in vitro* test system matches the *in vivo* situation in the ability to activate and to detoxify chemicals, the more predictive it will be for *in vivo* toxicity.

The rank order of toxicity of these chemicals, from most to least toxic, based on the LD_{50} values given in Table 13 is: DES > CH ≅ CY > AC. The LD_{50} value for DES was determined using DES dissolved in ethanol and the i.p. route of administration (27); the LD_{50} value for DES dissolved in dimethyl sulfoxide adminstered i.p. in the same strain of rat (Sprague-Dawley) was 100 mg/kg (27,28). In the present study, 500 mg/kg/day (1.86 mmol/kg/day) DES administered daily by gavage for 10 days resulted in 10% mortality in male F344 rats. Replacing the i.p. LD_{50} value of 34 mg/kg in Table 13 with an oral LD_{50} value greater than 500 mg/kg, the rank order of toxicity of these chemicals becomes: CH ≅ CY > AC > DES. This toxicity ranking compares favorably with the *in vivo* toxicity rankings, given in Table 12, based on toxicity observed after 10 days of repeated dosing.

Screening samples for biological activity and prioritizing samples for further *in vivo* evaluation are the functions of the proposed *in vivo* Prescreen. The present results demonstrate the potential usefulness of the CHO cytotoxicity assay to screen samples for biological activity and the ability of the assay to detect chemicals that are toxic *in vivo*, but raise a question regarding the potential usefulness of this assay to predict *in vivo* toxicity rankings. However, it should be recognized that all four chemicals that we evaluated are known human and animal toxins, that all four chemicals were found to be toxic in the *in vivo* toxicological screen described here, and that all four rank either as very toxic or extremely toxic when the ranking scheme given by Klaassen and Doull for human LD_{50} values is applied to the rat LD_{50} values in Table 13 (29). Thus the lack of correlation between the *in vivo* and the *in vitro* data in this study may not indicate a lack of ability to differentiate between non-toxic or weakly toxic and highly toxic compounds, but may rather indicate the inability to discriminate between highly toxic compounds. The small number of chemicals that we tested both *in vivo* and *in vitro* presently limits our ability to assess the usefulness of this assay.

TABLE 13.

Comparison of In Vitro Toxicity Rankings with Rat LD_{50} Data Using the Method of Garrett and Lewtas (8).

		In Vitro			In Vivo		
Chemical	Endpoint	EC_{50} (mM)	EC_{50} (ug/ml)	Ranking[2]	Rat LD_{50}[3] (mg/kg)	Route[4]	Ranking[3]
DES							
	VI[5]	0.04	10.7	moderate/high	34	i.p.	high
	ATP	0.03	8.0	high			
	Protein	0.03	8.0	high			
Chlordecone							
	VI	0.04	19.6	moderate	95	oral	high/ moderate
	ATP	0.02	9.8	high/moderate			
	Protein	0.02	9.8	high/moderate			
Acrylamide							
	VI	4.90	350	low	170	oral	moderate
	ATP	9.50	675	low			
	Protein	7.20	510	low			
Cyclophosphamide							
	VI	15.5	4330	N.D.	94		high/ moderate
	ATP	14.2	3960	N.D.			
	Protein	10.2	2900	N.D.			

1 We calculated the EC_{50} values in mM using the method of Garrett and Stack (30), then converted the EC_{50}'s from mM to ug/ml.

2 We used the toxicity rankings used by Garrett and Lewtas: high toxicity: < 10 µg/ml; moderate toxicity: 10-100 µg/ml; low toxicity: 100-1000 µg/ml; no detectable toxicity (N.D.): > 1000 µg/ml.

3 LD_{50} concentrations were taken from the Registry of the Toxic Effects of Chemical Substances. We used the toxicity rankings used by Garrett and Lewtas: high: < 100 mg/kg; moderate: 100-1000 mg/kg; low: 1-10 g/kg; no detectable toxicity (N.D.): > 10 g/kg.

4 While Garrett and Lewtas used only LD_{50} data for oral administration, we used data from i.p. administration when the oral LD_{50} was not given.

5 VI = Viability index.

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